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CHAPTER

2

Uncommon lineage switch warrants immunophenotyping even in relapsing leukemia

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Introduction

Despite intensive treatment, 60% of adult patients with T-cell acute lymphoblastic leukemia (T-ALL), experience a relapse of the disease [1]. In the majority of cases this is due to the outgrowth of the initial T-ALL clone from minimal residual disease [2]. However, the induction of secondary acute myeloid leukemia (AML) by previous chemotherapeutic treatment, such as etoposide, has been described in about 5–7% of the cases at relapse, generally more than 2 years after the initial diagnosis. In most of the cases specific chromosomal abnormalities are present, which were not present at diagnosis [3–4]. Alternatively, relapses with complete lineage switches can occur. This has been proposed to be the result of the outgrowth of an undifferentiated leukemic stem cell, the result of the preferential outgrowth of a minor AML subclone present at diagnosis or the result of therapy [5–10]. In general, the morphology of the leukemic cells at relapse suggests a switch from ALL to AML. The patient described here showed a complete immunophenotypic switch, which was not considered because of similar morphology and chromosomal abnormalities at relapse and at diagnosis. In view of the increasing use of monoclonal antibodies in the treatment of acute leukemia, immunophenotypic investigation of leukemic cells at diagnosis and relapse will be of importance in clinical practice [11–12].

Case

A 20-year-old man was admitted to the hospital with recurring and antibiotic-resistant paronychia. He experienced a 25-kg weight loss in the preceding 3 months and complained of night sweats and fever. His medical history was unremarkable. Physical examination revealed paronychia of two toes and enlarged tonsils. There were no signs of lymphadenopathy, hepatosplenomegaly nor gingival hyperplasia. Laboratory investigations revealed an elevated lactate dehydrogenase (690 U/l; normal values 0–250 U/l) and a decreased hemoglobin level (6.8 mmol/l; normal values 8.5–10.5 mmol/l). The total leukocyte count and platelet count was normal. In the differential count, 70% blasts were found. By light microscopy, the blast cells were stained negative for Sudan black with many hand mirror cells (Fig. 1A). Flow cytometric analysis showed blasts (CD34+) of lymphoid origin (cytCD3+, CD5+, CD2+, TdT+, CD7+, CD3–, CD1a–, CD10–, MPO–, CD33–, CD117–, CD19–), with aberrant expression of CD56 and weak expression of CD13 (Fig. 2A). Based on morphology and immunophenotyping the diagnosis of a precursor T-cell acute lymphoblastic leukemia (T-ALL) was made according to WHO criteria. Cytogenetic analysis of bone marrow cells revealed a complex karyotype 52, XY, +?X, +8, +10, +11, +13, +19 [11]; 46, XY [9]. FISH analysis performed with the LSI MLL-probe (Vysis) confirmed the presence of trisomy 11 by detecting 3 copies of the MLL-gene in 75% of 200 cells analysed. No molecular aberrancies could be detected by PCR. A lumbar puncture showed no localization of T-ALL. The patient was treated according to a current Dutch ALL protocol (HOVON 70; Fig. 3). As

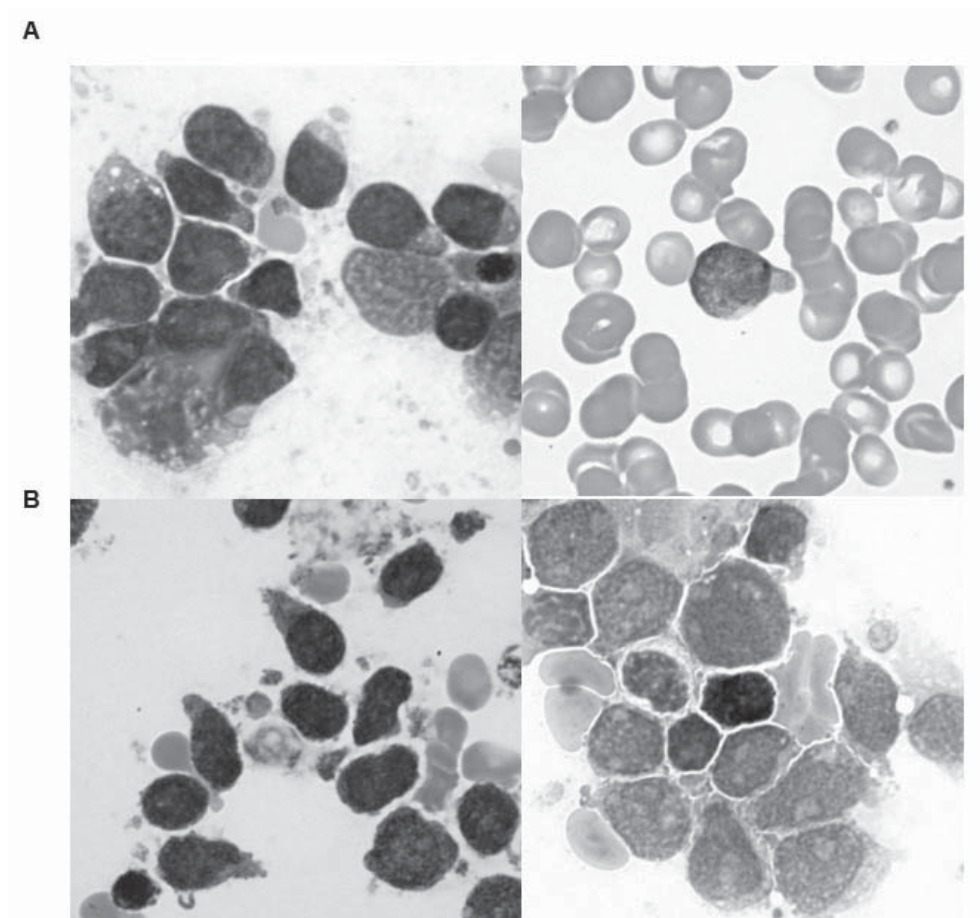


Figure 1. Morphology of leukemic cells at diagnosis and relapse. (A) Bone marrow and peripheral blood May-Gruenwald Giemsa (MGG) staining at diagnosis of T-ALL. (B) Bone marrow MGG and Sudan black staining of AML at relapse. This Figure is shown in color at the end of this thesis (p148).

no HLA-identical sibling was available he proceeded to maintenance therapy following induction and consolidation therapy, resulting in a complete remission. After nine months of maintenance therapy, 21 months after initial presentation, a relapse occurred. A bone marrow aspiration showed 91% Sudan black negative blasts with morphological features similar to diagnosis (Fig. 1B). Cytogenetic analysis showed an identical abnormal karyotype as compared at diagnosis. Molecular investigations (PCR) revealed no t(8;21) and FISH analysis showed trisomy 11 by MLL-gene analysis in 88% of the cells. Remarkably, the immunophenotype of the cells was completely switched from a lymphoid to a myeloid phenotype (CD117+, CD33+, CD13+, CD56+, MPO–, TdT–, CD7–, cCD3–, CD2–, CD5–, CD19+, Fig. 2B). AML was diagnosed following the WHO criteria. With the intention to perform an allogeneic stem cell transplantation with a matched unrelated donor, reinduction chemotherapy was initiated with high dose cytarabine (1000 mg/m² tid for 4 days) and etoposide

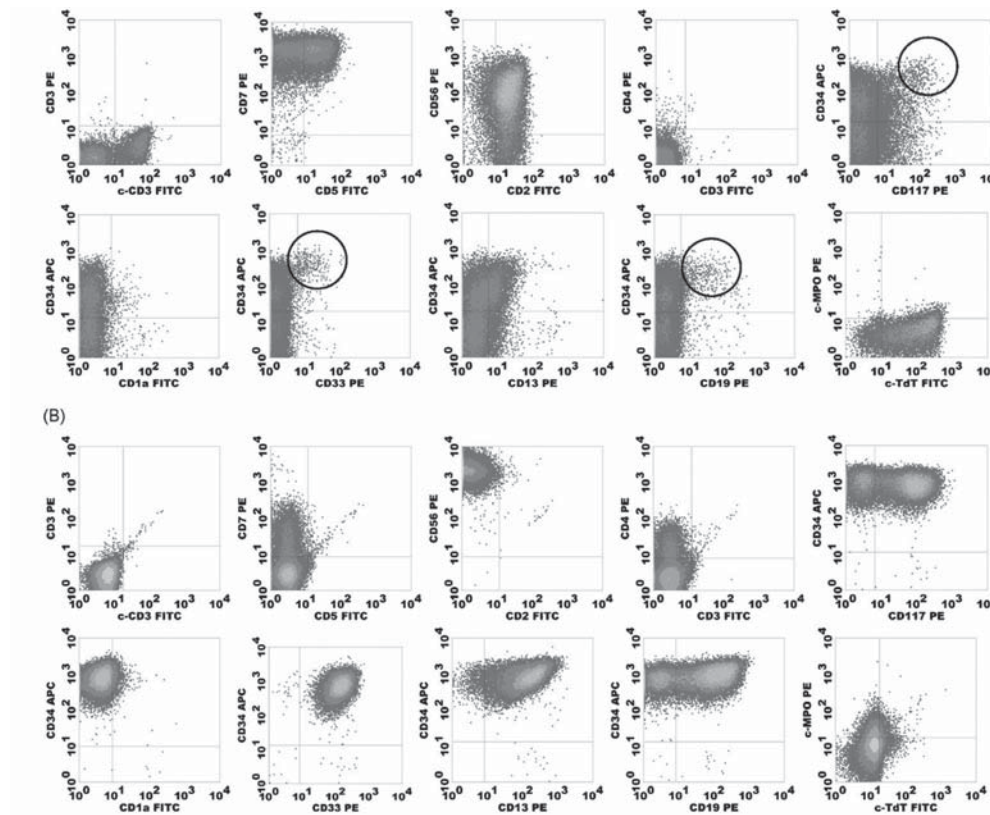


Figure 2. Immunophenotype of leukemic cells at diagnosis and relapse. (A) Immunophenotypic analysis of the T-ALL cells in bone marrow at diagnosis. A small myeloid subclone is encircled. (B) Immunophenotype of the myeloid cell population in peripheral blood at relapse.

(120 mg/m² oid for 4 days). Since no remission was reached, the patient subsequently underwent an autologous stem cell transplantation after treatment with high dose melphalan (200 mg/m²) [13]. Unfortunately, no remission was reached and he died 9 months after diagnosis of relapse due to the complications of longstanding pancytopenia.

Discussion

True lineage switches from T-ALL to AML in adolescents and adults are rare [5-7, 9, 14-16]. In a few of these cases, like in our case, both lymphoid and myeloid blast appeared to originate from one clone, as shown by an identical karyotype of the malignant cells [5-9].

Different hypotheses have been proposed for the occurrence of a lineage switch of clonally related cells in acute leukemia. It has been suggested that chemotherapy can induce changes in the differentiation program of the leukemic stem cell followed by switch of marker

Week 1	Pre-phase	Prednisone 60mg/m ²
Week 2-5	Induction	Prednisone 40mg/m ² ; Vincristine 1.5mg/m ² (max 2.0); Daunorubicin 40mg/m ² ; Cyclophosphamide 1000mg/m ² ; Asparaginase 6000IU/m ² ; Methotrexate intrathecal;
Week 5-9	Consolidation A	Thioguanine 60mg/m ² ; Cyclophosphamide 1000mg/m ² ; Cytarabine tid 30mg/m ² ; Methotrexate intrathecal
Week 10-13	Consolidation B	Prednisone 40mg/m ² ; 6-Mercaptopurine 50mg/m ² ; Vincristine 1.5mg/m ² (max 2.0); Methotrexate 5000mg/m ²
Week 14-17	Intensification IA	Dexamethasone 10mg/m ² ; Vincristine 1.5mg/m ² (max2.0); Adriamycine 25mg/m ² ; Asparaginase 6000IU/m ² ; Methotrexate intrathecal
Week 18-21	Intensification IB	Thioguanine 60mg/m ² ; Etoposide 150mg/m ² ; Cytarabine tid 30 mg/m ² ; Methotrexate intrathecal
Week 22-25	Interphase A	Prednisone 40mg/m ² ; Vincristine 1.5mg/m ² (max 2.0); 6-Mercaptopurine 50mg/m ² ; Methotrexate 5000mg/m ²
Week 26-29	Interphase B	Prednisone 1dd 40mg/m ² ; 6-mercaptopurine 50mg/m ² ; Vincristine 1.5mg/m ² (max 2.0); Methotrexate 5000mg/m ²
Week 30-33	Intensification IIA	Prednisone 40mg/m ² ; Vincristine 1.5mg/m ² (max2.0); Daunorubicin 30mg/m ² ; Asparaginase 6000IU/m ² ; Methotrexate intrathecal
Week 34-37	Intensification IIB	Thioguanin 60mg/m ² ; Cyclophosphamide 1000mg/m ² ; Cytarabine tid 30mg/m ² ; Methotrexate intrathecal
Week 38-104	Maintenance therapy	6-mercaptopurine 75mg/m ² (daily); Methotrexate 25mg/m ² (weekly)
Week 38-104	Re-induction	Prednisone 40mg/m ² (monthly); Vincristine 1.5mg/m ² (max2.0) (monthly)

Figure 3. ALL treatment according to the Dutch standard protocol HOVON 70.

expression, while retaining its cytogenetic aberrancies [15, 17]. Interestingly, the group of Kurtzberg et al. described the occurrence of a lineage shift already 7 days after chemotherapy treatment with adenosine deaminase inhibitor 2'-deoxycytosine. Because of the immature lymphoid phenotype (CD7+, CD4- and CD8-) and the rapid lineage switch, they hypothesized that the patient had a leukemia of a pluripotent stem cell that was capable of multilineage differentiation [8]. This outgrowth of an undifferentiated stem cell clone, could be confirmed in eight patients, from whom in vitro cultures of leukemic blasts gave rise to morphologically and immunophenotypically distinguishable progenitors of the myeloid, erythroid, megakaryocytoid, eosinophil, monocytoid, and lymphoid lineages independent of exposure to exogenous growth factors [18]. This is supported by the finding that in T-cell leukemias with a CD7+, CD4-, CD8- and CD1- phenotype a stem cell phenotype can be determined defined as absence of both cCD3 and MPO expression [19]. Additionally, in some of these patients a mixed lineage cell type could be determined, expressing both cyCD3 and MPO. Accordingly, more recently it has been described that early thymic progenitor cells possess both T-cell as well as myeloid differentiation potential [20-22]. After discovery of this early immature phenotype (CD7+, CD4-, CD8- and CD1-) in ALL, these cases were classified as immature T-ALL or T-stem cell leukemia [23]. Our patient also showed an immature T lymphoid phenotype: CD7+, CD4-, CD8- and CD1a- at initial diagnosis. At relapse

this changed into an immature myeloid phenotype: CD34+, CD117+, CD13+, CD33+, CD19+, MPO–, and CD7–. Therefore, also in our case it can be hypothesised that under pressure of anti-lymphoid-leukemia directed therapy, an undifferentiated stem cell clone grew out as a myeloid relapse. On the other hand, in retrospect, we found a small population of CD34high cells of 1.2% in the CD45dim population at initial presentation (with possible co-expression of CD117, CD33 and CD19).

— 48

This population differed from the normal pre-B cells, which were brighter CD19 positive, lower in their CD34 expression and low in forward/side scatter compared to the CD34high population. Consequently, it can also be considered that a malignant population of myeloid blasts was already present at initial presentation (Fig. 2A). Unfortunately, due to lack of material we were not able to classify this small population as myeloid malignant at diagnosis and therefore as bi-linear. In bi-lineage leukemias the therapy would have been directed towards the major population. Since the detected population in our patient covered only 1% of the total amount of blasts, the therapy would have been similar. However, in case of bi-linear leukemia, regular immunophenotyping to determine the immunophenotype of residual disease might indicate the need for therapy switches. A recent case of AML which relapsed as a T-ALL was described in which evidence was gained for a small T-cell clone at initial presentation with similar cytogenetic aberrancies.

In conclusion, we showed that a complete switch of immunophenotype can occur, while both morphology and cytogenetic features remain identical to the disease at diagnosis. In view of the increasing use of monoclonal antibodies this will be of importance in clinical practice. In this case anti-CD33 could have been an option in complementing standard high doses chemotherapy in conditioning treatment before unrelated matched stem cell transplantation. This case emphasizes the clinical importance of flow cytometric analysis at any relapse in patients with acute leukemias.

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